bjh review

Parvovirus transmission by blood products – a cause for concern?

Päivi Norja,[1] Riitta Lassila[2] and Mike Makris[3,4]

[1]Department of Virology, Haartman Institute, Helsinki University, [2]Coagulation disorders Unit, Division of Haematology, Department of Internal Medicine, Helsinki University Central Hospital, Helsinki, Finland, [3]Department of Cardiovascular Science, University of Sheffield, and [4]Sheffield Haemophilia and Thrombosis Centre, Royal Hallamshire Hospital, Sheffield, UK

Summary

The introduction of dual viral inactivation of clotting factor concentrates has practically eliminated infections by viruses associated with significant pathogenicity over the last 20 years. Despite this, theoretical concerns about transmission of infection have remained, as it is known that currently available viral inactivation methods are unable to eliminate parvovirus B19 or prions from these products. Recently, concern has been raised following the identification of the new parvoviruses, human parvovirus 4 (PARV4) and new genotypes of parvovirus B19, in blood products. Parvoviruses do not cause chronic pathogenicity similar to human immunodeficiency virus or hepatitis C virus, but nevertheless may cause clinical manifestations, especially in immunosuppressed patients. Manufacturers should institute measures, such as minipool polymerase chain reaction testing, to ensure that their products contain no known viruses. So far, human bocavirus, another new genus of parvovirus, has not been detected in fractionated blood products, and unless their presence can be demonstrated, routine testing during manufacture is not essential. Continued surveillance of the patients and of the safety of blood products remains an important ongoing issue.

Keywords: haemophilia, coagulation, parvovirus, parvovirus 4, clotting factor concentrate.

Patients with inherited bleedings disorders, such as severe haemophilia A, B, von Willebrand disease (VWD), as well as other rare bleeding disorders suffer recurrent spontaneous and traumatic bleeds and are treated with intravenous infusions of the missing clotting factor, so-called replacement therapy.

The treatment of bleeding disorders has developed enormously from the use of fresh frozen plasma in the 1940s, cryoprecipitate in the 1960s, and clotting factor concentrates since the early 1970s. Over the last 20 years recombinant technology, aimed at avoidance of all animal- or human-derived proteins during the manufacture or final formulation of the coagulation factor concentrates, has been developed. Until recently, the major drive in concentrate development has been the reduction in infective risk. Today, the development of allo-antibodies to factor VIII (FVIII) (inhibitors) has taken over as the main problem in haemophilia management.

Plasma-derived clotting factor concentrates are prepared by fractionation of up to 30 000 pooled plasma units. Until viral inactivation was introduced in 1985, viral infections present in the donors could easily be transmitted to the recipient. The result was that virtually all recipients of concentrates prior to 1985 were infected with hepatitis C virus (HCV) and many were also infected with human immunodeficiency virus (HIV) and hepatitis B virus (HBV) (Makris *et al*, 1996). The viral elimination processes are based on destruction of the viruses with dry or wet heat treatment (sometimes under pressure), chemical treatment with combination of solvent and detergent (S/D), and nanofiltration (Mannucci & Tuddenham, 2001).

Viral elimination processes proved to be highly successful in virtually abolishing the risk of infection with HBV, HCV and HIV. However, in 1992, a number of outbreaks of hepatitis A transmission by concentrates were reported (Richardson & Evatt, 2000). This occurred due to the fact that hepatitis A, which does not have a lipid envelope, was resistant to the viral elimination by the S/D method used during manufacture. Subsequently, new regulations require that all plasma-derived clotting factor concentrates undergo two different viral elimination procedures before release.

Despite the success of the currently used viral elimination techniques, two infectious agent problems have remained, parvovirus B19 (B19) and prion transmission. As B19 is relatively resistant to all the currently available elimination methods, manufacturers introduced screening of mini-pools by the polymerase chain reaction (PCR). Positive minipools are not used in fractionation of blood products, but despite this, recent evidence suggests that the risk of B19 transmission is

Correspondence: Dr Mike Makris, Department of Cardiovascular Science, University of Sheffield, Royal Hallamshire Hospital, Sheffield S10 2JF, UK. E-mail: m.makris@sheffield.ac.uk

First published online 1 October 2012
doi:10.1111/bjh.12060

real (Soucie *et al*, 2011). Variant Creutzfeld Jacob Disease (vCJD), a prion disease, is the human form of the Bovine Spongiform Encephalopathy (BSE) that appeared in the UK cattle population in the 1980s. Transmission by blood products and clotting factor concentrates has been demonstrated (Peden *et al*, 2010). Prions are highly resistant to all currently used elimination techniques but because of geographical restriction of vCJD, some countries have chosen to avoid using plasma collected in those countries, e.g., the UK (Millar *et al*, 2010).

Further viral newcomers in this field include human parvovirus 4 (PARV4), which has been linked with intravenous drug administration, both for therapeutic use, such as for bleeding disorders, and recreational abuse. In recognition of the difficulty of eliminating infective agents from clotting factor concentrates, some countries (e.g., UK, Canada and Ireland) decided to use exclusively recombinant clotting factors, when these became available. This can be envisioned as protectionism in the countries where this kind of national transitions have not been undertaken. The current plasma-derived products have proven safe, at least so far.

Our objective here is to illustrate that, in association with intravenous repetitive coagulation factor replacement therapies – despite the current precautions – the risk of viral transmittance cannot be completely excluded. The surveillance of both old and new patients and concentrates remains our continued task.

We live in a world where new disease entities and viral epidemics continue to emerge in various locations and, with the current air travel frequency, these diseases can spread quite rapidly. The recently identified west Nile virus and swine influenza are examples of suddenly emerging pathogenic viruses that have a relatively strong penetrance and cause local and even worldwide epidemics. Additionally, prions are an example of pathogens, which are very hard to detect and the success of their elimination remains unclear for many years, with potentially serious consequences.

Parvovirus taxonomy and basic features

The name parvovirus originates from the Latin word *parvum,* which means small; parvoviruses are among the smallest known viruses with a virion diameter of 18–26 nm. Parvoviruses infect a wide range of vertebrates and insects causing systemic infections. The family *Parvoviridae* is divided into two sub-families: Parvovirinae and Densovirinae (http://ictvonline.org/virusTaxonomy.asp?version=2009). Viruses from the sub-family Parvovirinae infect vertebrate cells and are divided further into five genera (Table I): *Parvovirus, Dependovirus, Erythrovirus, Bocavirus,* and *Amdovirus*. Of these, *Erythrovirus*, *Dependovirus* and *Bocavirus* genera contain viruses infecting humans. In addition, a sixth genus, *Partetravirus,* containing human PARV4 and human PARV4-like viruses, has been proposed. The sub-family of Densovirinae contains viruses of invertebrates.

Table I. The taxonomy of parvoviruses.

Subfamily	Genus	Example virus, abbreviation
Parvovirinae	*Parvovirus*	*Minute virus of Mice, MVM*
		Canine parvovirus, CPV
	Dependovirus	*Adeno-assosiated virus AAV*
		Goose parvovirus
	Erythrovirus	*Human parvovirus, B19*
		Simian parvovirus, SPV
	Bocavirus	*Bovine parvovirus, BPV*
		Human bocaviruses 1-4, HBoV1-4
	Amdovirus	*Aleutian mink disease virus, AMVD*
	Partetravirus	*Human partetravirus, PARV4*
	(proposed)	*Porcine hokovirus, PHoV/PPV3*
Densovirinae	*Densovirus*	*Junonia coenia densovirus*
	Brevidensovirus	*Aedes aegypti densovirus*
	Iteravirus	*Bombyx mori densovirus*
	Pefudensovirus	*Periplanta fuliginosa densovirus*

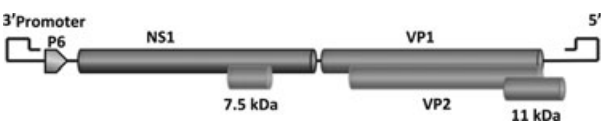

Fig 1. Genome structure and protein encoding reading frames of B19.

The structure of parvoviruses is simple; the icosahedral virion consists of only proteins and linear single-stranded-DNA genome with hairpin structures at both ends. The hairpins are palindromic and the 3′-end can fold and function as a primer during viral replication (Fig 1). The length of the DNA genome is approximately 5–6 kb. In the parvovirus infection cycle, the virus attaches to its receptor, e.g. globoside (P-antigen) in case of human B19, on the surface of host cells (Brown *et al*, 1993) and is transported into the cell by endocytosis. Inside the host cell, the virion is transported to the nucleus where parvovirus replication takes place. Parvoviruses do not encode their own DNA-polymerase, indicating that all parvoviruses are dependent on (i) host cell polymerase and (ii) S-phase of dividing cells. In the case of dependoviruses, co-infection with another virus is needed for efficient DNA replication (Atchison *et al*, 1965).

General aspects and epidemiology of human and porcine parvoviruses

Parvovirus B19

Human parvovirus B19 (B19) is the type species of the *Erythrovirus* and representative member of parvoviruses. B19 was discovered when a serum sample from an asymptomatic blood donor gave a false-positive result in an immunoelectrophoresis assay for HBV (Cossart *et al*, 1975). The virus was detected in panel B and was coded 19, from which its name originates. The most common transmission route of B19 is respiratory, but it can also transmit via

plasma-derived medical products and from mother to fetus. B19 infections are prevalent worldwide, and seroprevalence studies based on B19 IgG have shown that in Europe 60–80% of adults have been infected with this virus during their lifetime (Mossong *et al*, 2008). In Asia the B19 seroprevalence in blood donors was found to be 25–40% (Kishore *et al*, 2010, Ke *et al*, 2011).

B19 DNA prevalence in blood donors has been reported by a number of studies. The rates of positivity were 0·88% in the USA (Kleinman *et al*, 2007), 0·2% in the Netherlands (Koppelman *et al*, 2011) and 0·55–1·3% in the UK and Africa (Candotti *et al*, 2004).

B19 is erythrotropic and replicates in erythroid progenitor cells in human bone marrow. After short viraemia the virus is eliminated from the blood circulation by neutralizing antibodies produced by the host. However, B19 genomic DNA remains detectable in solid tissues of seropositive individuals. Initially, B19 DNA was detected in the synovium of patients with rheumatoid arthritis but, in 1997, the viral DNA was also detected in 48% of synovia collected from healthy controls (Saal *et al*, 1992; Soderlund *et al*, 1997). Later, B19 was shown to persist with full-length coding capacity in several tissue types of both symptomatic and asymptomatic persons, most probably for a lifetime (Soderlund-Venermo *et al*, 2002; Norja *et al*, 2006; Manning *et al*, 2007).

B19 strains have been divided into three divergent genotypes according to their genomic sequence. Genotype 1 is the prototypic virus and is nowadays globally the most predominant circulating virus (Hubschen *et al*, 2009). The genotype 2 virus was first identified in human skin and in the serum of an Italian HIV-positive patient with chronic anaemia (Hokynar *et al*, 2002; Nguyen *et al*, 2002). Genotype 2 has since been found in human solid tissues but only sporadically in blood and seems to have disappeared from wide circulation after the 1970s (Blumel *et al*, 2005; Norja *et al*, 2006; Manning *et al*, 2007; Grabarczyk *et al*, 2011, Koppelman *et al*, 2011). The genotype 3 virus was found in France in the serum and bone marrow of a child with transient aplastic anaemia (Nguyen *et al*, 1999). Following its discovery, genotype 3 has been reported to be endemic in Ghana and Brazil (Candotti *et al*, 2004; Sanabani *et al*, 2006; Freitas *et al*, 2008; Keller *et al*, 2009). Since identification of the genotypes 2 and 3, many commercial and in-house PCR-methods have been shown to detect these B19 genotypes with lower sensitivity or fail to detect either or both of these genotypes (Hokynar *et al*, 2004; Baylis, 2008).

Bocavirus

Human bocavirus 1 (HBoV1) was first identified in 2005 by random molecular screening and large-scale sequencing. HBoV1 was discovered in Sweden, in nasopharyngeal aspirates of children with respiratory tract infections (Allander *et al*, 2005). It belongs to the genus *Bocavirus* and its closest relatives are the bovine parvovirus (BPV) and the minute virus of canines (MVC). The receptors and target cells of HBoV1 are unknown and, to date, HBoV1 has been cultured only in pseudo-stratified human airway epithelium cell culture system (Dijkman *et al*, 2009). Using similar methods of random amplification, three additional human bocaviruses were identified in faecal samples in 2009 and 2010 (Arthur *et al*, 2009; Kapoor *et al*, 2009, 2010). These new HBoVs were named HBoV2, HBoV3 and HBoV4. Of these HBoV2 seems to be the most prevalent and circulates globally (Arthur *et al*, 2009; Kapoor *et al*, 2009, 2010; Chow *et al*, 2010; Kantola *et al*, 2010).

The seroprevalence of HBoV1 has been reported to be more than 90% in adults. However, the HBoV1-4 viral-like particles used in the enzyme-linked immunosorbent assay (ELISA) have been shown to cross-react, which might affect the serological assays. Norja *et al* (2012) detected an HBoV1 seroprevalence of 94·9%, but after removing cross-reacting antibodies the rate was 68·4%. Similar results were obtained by Kantola *et al* (2011), who observed that adult HBoV1 seroprevalence decreased, from 96 to 59%, after removing the cross-reacting antibodies. The Kantola study reported HBoV2-4 seroprevalences among adults of 34% for HBoV2, 15% for HBoV3, and 2% for HBoV4 (Kantola *et al*, 2011). As far as we are aware, human bocavirus DNA has not been detected in blood donations.

Parvovirus 4

PARV4 was identified in 2005 in a HBV-positive intravenous drug abuser with various viral infection-related symptoms by similar methods to the HBoVs (Jones *et al*, 2005). During the following year, a related virus variant (PARV5) was identified in plasma pools used in the manufacture of plasma-derived medicinal products (Fryer *et al*, 2006). Subsequently, the name PARV5 was changed to PARV4 genotype 2 (Fryer *et al*, 2007a). In 2008, a third genotype of PARV4 was identified in HIV-infected African patients (Simmonds *et al*, 2008). DNAs for PARV4 genotype-1 and -2 have been found in bone marrow, lymphoid tissue, and liver of subjects with a history of intravenous drug use, or HIV, or HCV infection (Manning *et al*, 2007; Simmonds *et al*, 2007; Longhi *et al*, 2007; Schneider *et al*, 2008a). In addition, several studies described PARV4 DNA in donor blood samples and coagulation factor concentrates (Fryer *et al*, 2006, 2007a,b; Lurcharchaiwong *et al*, 2008; Schneider *et al*, 2008b). Initially the parenteral transmission route was proposed for PARV4, but the genotype 3 of PARV4 has also been found in subjects without a risk of parenteral exposure (Simmonds *et al*, 2008; Panning *et al*, 2010).

Porcine parvovirus

Porcine parvovirus (PPV1) was first isolated in Germany and the USA in 1965 and today it is found worldwide

(Csagola *et al*, 2012). PPV1 belongs to the genus *Parvovirus*. PPV1 is transmitted oronasally among seronegative dams (female parents) and the virus is then further transmitted through the placenta to fetus, causing reproductive failure. During the last decade, several new parvoviruses have been identified in pigs, including porcine hokovirus (PHoV/PPV3), which is related to PARV4 (Csagola *et al*, 2012).

Human diseases caused by parvoviruses

Until the discovery of HBoVs and PARV4, human B19 was considered to be the only pathogenic parvovirus to humans. The adeno-associate viruses of *Dependovirus* genus are non-pathogenic and are studied as vectors for gene-therapy. Although B19 is associated with various clinical manifestations, subclinical infection is a common finding among both children and adults. In healthy, immunocompetent subjects, B19 infection is usually mild and transient, requiring no treatment.

The most common B19 manifestation among children is a rash causing the disease *Erythema infectiosum* (EI), fifth disease or 'slapped cheek', and arthritis among adults (Anderson *et al*, 1984). In EI, the rash typically appears first on the cheeks, spreading to the neck, trunk, and limbs. In addition, the patient may have headache, fever, nausea, and diarrhoea. Among adults, arthritis can be the only manifestation of B19 infection, affecting 45–80% of infected subjects (Anderson *et al*, 1985; Reid *et al*, 1985; White *et al*, 1985). Joint symptoms are symmetrical and affect fingers, wrists, ankles, and knees. Arthritis is usually transient but in some cases it may be prolonged and fulfill the criteria of rheumatoid arthritis (Naides *et al*, 1990).

Women without B19-specific antibodies are at risk of primary B19 infection and trans-placental transmission. During maternal infection, the risk of vertical transmission is approximately 30% (Brown, 2010). Intrauterine B19 infection has been associated with fetal anaemia, hydrops, miscarriage, and fetal death (Enders *et al*, 2006, 2008).

In subjects with shortened red-cell survival, such as sickle cell disease, B19-infection may lead to aplastic crisis (Pattison *et al*, 1981). Among immunosuppressed subjects with decreased ability to produce antibodies, including patients with leukaemia or lymphoma (Kurtzman *et al*, 1988, 1989) or in the HIV/HCV-infected, the B19 infection may become persistent causing chronic anaemia.

Following the HBoV1 discovery, a large number of studies of the prevalence of HBoV1 have been undertaken in respiratory secretions of young children. According to recent studies, primary infections of HBoV1 are significantly associated with respiratory illnesses, including wheezing, pneumonia, and otitis media (Soderlund-Venermo *et al*, 2009; Don *et al*, 2010; Meriluoto *et al*, 2012). HBoV1 has also been detected in faeces from children with symptoms of gastroenteritis. However, the significance of HBoV1 as an enteric virus is questionable, because in many subjects another enteric virus was detected simultaneously with HBoV1, and there is a lack of evidence of replication of HBoV1 in the enteric tract (Albuquerque *et al*, 2007; Yu *et al*, 2008, Szomor *et al*, 2009). HBoV2 instead may cause gastroenteritis in young children (Kapoor *et al*, 2009; Chow *et al*, 2010; Kantola *et al*, 2010).

So far, no disease associations have been confirmed for PARV4 (Lahtinen *et al*, 2011). The virus has been linked to encephalitis (Benjamin *et al*, 2011), and detected in the blood of three mothers bearing newborns with hydrops (Chen *et al*, 2011). Among patients with haemophilia, clinical presentations concurrent with PARV4 seroconversion were rash and unexplained hepatitis (Sharp *et al*, 2012). The individual in whom PARV4 was first identified presented with fatigue, vomiting, arthralgia, neck stiffness, night sweats, and diarrhoea, but this patient was lost to follow up, and it is not known if the described symptoms were associated with the PARV4 infection (Jones *et al*, 2005).

There are no antiviral drugs or vaccines against human parvovirus infection. However, among immunocompetent patients, treatment is unnecessary and infections are self cleared. Immunodeficient patients with chronic B19 infection, and patients with transient aplastic anaemia and B19, can be managed with intravenous immunoglobulin or erythrocyte transfusions (Frickhofen *et al*, 1990; Koduri *et al*, 1999).

Parvoviruses in blood products

Parvovirus B19

The B19 titre in blood is at its highest, up to 10^{13} genome equivalents/ml blood, during the first days of acute infection. Infected subjects are usually asymptomatic when the viral titres are at their highest. This creates a risk of contaminating blood products by blood donors with asymptomatic B19 infection. Siegl and Cassinotti (1998) reported B19 DNA in 50–80% and in 30–50% of non-virally inactivated VIII concentrates and S/D-inactivated coagulation factor IX (FIX) concentrates, respectively. A more recent German study detected B19 DNA in 26% of coagulation factor concentrates of different types, collected between 2007 and 2008 (Modrow *et al*, 2011). The highest viral loads were observed in the intermediate purity FVIII /VWF concentrates. Because of its small and non-enveloped structure, B19 is relatively resistant to most viral inactivation procedures used in the manufacturing of medical blood-derived products (Willkommen *et al*, 1999; Koenigbauer *et al*, 2000; Schmidt *et al*, 2001) and B19 is only partially removable with small pore size nanofiltration (Burnouf-Radosevich *et al*, 1994).

In Europe, in an attempt to reduce the risk of B19 transmission by blood products, the blood derived products manufactured after 2004 are not allowed to contain B19 DNA of more than 10^4 iu/ml, and nucleic acid testing for B19 is

obligatory for S/D-treated human plasma products (European Pharmacopoeia Commission, Council of Europe European Directorate for the Quality of Medicines, 2011). Similar instructions are given by United States Food and Drug Administration (http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/default.htm). B19 DNA concentrations below the 10^4 iu/ml limit are not considered to lead to seroconversion (Brown *et al*, 2001). However, Soucie *et al* (2011) reported 1·7 times higher B19 IgG seroconversion rates among children who received plasma-derived factor concentrates screened for B19 levels than among children receiving recombinant products. The infectivity of B19 in the blood products is affected by both the level B19-specific IgG in the products and the immune status of the recipient.

In order to quantify the B19 positive units, the quantitative DNA amplification method is used, and primers designed to detect all three genotypes are required. Many in-house PCR methods and one commercial PCR method, designed before the identification of B19 genotypes, are prone to miss B19 genotypes 2 and 3 (Hokynar *et al*, 2004; Baylis *et al*, 2004; Koppelman *et al*, 2004, 2007). Consequently, some plasma pools have remained contaminated with an excessive level of B19. All three genotypes of B19 have been reported in clotting factor concentrates, however, with a reduced frequency for genotypes 2 and 3 (Schneider *et al*, 2004; Modrow *et al*, 2011). To obtain an accurate diagnosis and safety of blood products, methods with capability to detect all B19 genotypes should be used. A reference panel for B19 DNA genotypes by the World Health Organization Expert Committee on Biological Standardization (ECBS) was established at the end of 2009, and it is available for validation of B19 PCR-based detection assays for all three genotypes (Baylis *et al*, 2012). Furthermore, the most recent study of B19 levels in plasma donations described a commercial method for simultaneous B19 and HAV screening (Koppelman *et al*, 2012).

Human bocaviruses

Three studies have analysed whether HBoV1 occurs in blood donor plasma and plasma-derived coagulation factor concentrates (Fryer *et al*, 2007b; Eis-Hubinger *et al*, 2010; Modrow *et al*, 2011). None of the studies reported positive cases of HBoV1. The absence of detectable HBoV1 DNA in blood or plasma donors, however, may be due to the fact that HBoV1 infections are most common among young children (Soderlund-Venermo *et al*, 2009; Meriluoto *et al*, 2012) and less frequent among the blood donor population. Negative results could also be explained by low HBoV1 titres in donors, as possible low-level viraemia could remain undetectable in manufactured plasma pools containing hundreds or thousands of donations.

PARV4 DNA in blood-derived medical products

The first study of PARV4 in blood products was published soon after the virus was discovered and reported PARV4 DNA with prevalence of 5·1% in manufacturing plasma pools (Fryer *et al*, 2006). In this study both PARV4 genotype 1 and 2 DNAs were amplified, and the virus titres ranged between < 500 copies to 10^6 copies/ml. A year later, the same authors reported PARV4 genotype 1 and 2 DNAs in 4% of recently sourced plasma pools collected in Europe and the USA, in 21% of the older plasma pools collected between 1990 and 1993, in 2% of the blood collected from healthy blood donors and in 6% of febrile patients (Fryer *et al*, 2007b). Schneider *et al* (2008a) reported PARV4 in 1–33% of randomly selected plasma-derived concentrate pools. The higher frequency of the PARV4 was detected in the older concentrates manufactured 10 years earlier, but smaller amounts of PARV4 were also detectable in the currently used concentrates. Recently, a study from China reported PARV4 DNA in the blood of 16–22% healthy subjects, in 33% of HBV-infected subjects and in 41% of HCV-infected subjects (Yu *et al*, 2012). In addition, PARV4 DNA has been detected in 26% of blood donor plasma pools collected between 2007 and 2010 in China (Ma *et al*, 2012). Table II summarizes PARV4 DNA detected in plasma- and blood-derived medical products. Even if the disease associations of PARV4 are not currently known, the prevalences of PARV4, especially those detected most recently in France and China, raise a question of whether blood donor minipools should be tested by screening for PARV4 DNA similar to B19.

In contrast to the studies described in Table II, three studies performed in France and Germany analysed high numbers of blood donor plasma donations, minipools or coagulation factor concentrates and failed to detect any

Table II. PARV4 DNA findings in blood donor samples and coagulation factor concentrates.

References	Blood product	PARV4 DNA prevalence (%)
Fryer *et al* (2006)	Plasma pools	5·1
Fryer *et al* (2007a,b)	Plasma pools,	8·7
	individual plasma	4
Fryer *et al* (2007c)	FVIII concentrates	16
Lurcharchaiwong *et al* (2008)	Blood donor sera	3·95
Schneider *et al* (2008b)	Coagulation FFII, FVIII, FIX, activated prothrombin complex concentrates	21
Vallerini *et al* (2008)	Blood donor sera	1
Touinssi *et al* (2010)	Blood donor plasma	24·6
Ma *et al* (2012)	Plasma pools	26

PARV4 DNA positive samples (Servant-Delmas *et al*, 2009; Eis-Hubinger *et al*, 2010; Modrow *et al*, 2011). Whether these negative results are due to seasonal or geographical reasons need further studies. The unanswered question also is, whether the higher frequency of PARV4 in older blood products (Fryer *et al*, 2007a,b; Schneider *et al*, 2008b) represents a timely population-based hazard or whether the manufacturing processes, i.e., nanofiltration, have improved the elimination of the viruses more comprehensively. On the other hand, demonstration of virus genome in plasma-derived products does not translate to infectivity.

PARV4 and haemophilia

In a study of 35 persons with haemophilia from the UK and USA receiving replacement therapy, 15/35 (43%) were positive for the PARV4 IgG whereas only 1/35 (3%) of untreated family members were positive (Sharp *et al*, 2009). The concentrates involved in treatment were non-virally inactivated clotting factors issued from the late 1970s to the early 1980s. The methods of detection were serological, ELISA-type assays, detecting both anti-PARV4 IgG and IgM, developed by the group of Simmonds.

In a 5-year follow-up of a cohort of 194 haemophilia patients who were born between 1972 and 1982, 1–7% of patients/year seroconverted for PARV4 (Sharp *et al*, 2012). They were followed between 1989 and 1994 by 6 monthly blood sampling. At cohort enrolment, almost all patients were HCV-positive and 43% of patients were PARV4 IgG-positive. Among PARV4 seropositive subjects, 46% were HIV-positive and 38% HIV-negative, thus PARV4 exposure did not significantly associate with HIV infection. The active disease forms related to PARV4 detection were rash and exacerbation of hepatitis. PARV4 IgM became positive during acute infections. The concentrates involved were plasma-derived and had undergone S/D treatment and dry or wet heating processes. Overall, the seroprevalence and the risk of seroconversion are significantly higher in patients having replacement therapy than the background population or sibling controls.

Transmission of porcine parvovirus by the old Hyate C porcine concentrate

A serious complication of therapy of persons with haemophilia is the development of antibodies (inhibitors) against the clotting factor, which renders the concentrates ineffective in controlling bleeding. Porcine FVIII concentrate, Hyate C, has been used as a treatment of patients with congenital haemophilia and inhibitory antibodies. Hyate C was developed in 1980 and was manufactured by Ipsen Ltd (Slough, UK) from pig plasma. During the manufacturing process Hyate C underwent a number of purification steps, and cell culture was used to confirm the absence of viruses but, in contrast to human coagulation factor concentrates, it did not undergo viral inactivation. In 1996, PPV1 was found in several Hyate C products and its supply was suspended. The knowledge that many recipients of Hyate C were already infected by HIV and were immunosuppressed led to concerns that PPV1 could in some cases infect humans. Soucie *et al* (2000) detected PPV1 DNA in 95% of porcine FVIII concentrates and confirmed that PPV1 is a common, low level contaminant in Hyate C. However, none of the 98 recipients of Hyate C tested positive for PPV1 antibodies. Most pigs naturally have antibodies to PPV1, but there is no evidence of transmission to humans from physical contact between pigs and humans. In addition to PPV1, porcine hokovirus, closely related to PARV4, has recently been found in porcine plasma and FVIII preparations (Szelei *et al*, 2010). The theoretical risk that porcine parvoviruses could infect humans remains a concern, but, if employed, PCR screening and discarding the porcine parvovirus DNA positive samples could eliminate the risk of transmission. Porcine plasma-derived FVIII concentrates are no longer available and to our knowledge are not being developed but a recombinant porcine FVIII concentrate has recently started clinical trials.

Implications

New viruses and other disease-inducing agents will always continue to evolve. In the first 10 months of 2010 there were 2350 reports of outbreaks of infectious diseases in humans, plants and animals (www.promedmail.org). It must be appreciated that the identification of new infections internationally is the norm rather than the exception. The studies of PARV4 and haemophilia have shown that the virus can be transmitted via blood donations and plasma products, at least when the viral inactivation steps include the methods of S/D and heating. We do not know the infectivity frequency following nanofiltration, but it may eliminate the majority of the viral load (Schneider *et al*, 2008b). To date, the parvoviruses have not been proven to cause significant chronic pathogenesis in patients with a healthy immunological system. However, in patients already infected with HIV or otherwise immunocompromised, B19 has pathogenic consequences. Overall, the current data imply that viruses are able to escape the current plasma fractionation and purification steps.

For infections that are potentially transmissible by clotting factor concentrates, blood donors should be screened serologically and mini-pools of plasma should be genotyped with the virus load measured with PCR (EMA, 2010). The safety of the plasma-derived concentrates demands continuous watchful strategies and surveillance. The regulatory studies required for registration occur early in the introduction of the products onto the market and are not optimal at detecting infection transmission by agents other than hepatitis A/B/C, HIV and Parvovirus where acute seroconversion detection is possible. Continuous vigilance by the haemophilia community is required to identify infective problems

early. Adverse event reporting studies, such as the European Haemophilia Safety Surveillance (EUHASS) system, or national spontaneous reporting schemes have the potential to identify problems, but alertness to new or unusual problems is required for unexpected events (Makris *et al*, 2011). In this way any unexpected clinically significant transmission of infection by plasma products can be traced and eliminated as rapidly as possible.

References

Albuquerque, M.C., Rocha, L.N., Benati, F.J., Soares, C.C., Maranhao, A.G., Ramirez, M.L., Erdman, D. & Santos, N. (2007) Human bocavirus infection in children with gastroenteritis, Brazil. *Emerging Infectious Diseases*, **13**, 1756–1758.

Allander, T., Tammi, M.T., Eriksson, M., Bjerkner, A., Tiveljung-Lindell, A. & Andersson, B. (2005) Cloning of a human parvovirus by molecular screening of respiratory tract samples. *Proceedings of the National Academy of Sciences of the United States of America*, **102**, 12891–12896.

Anderson, M.J., Lewis, E., Kidd, I.M., Hall, S.M. & Cohen, B.J. (1984) An outbreak of erythema infectiosum associated with human parvovirus infection. *The Journal of Hygiene*, **93**, 85–93.

Anderson, M.J., Higgins, P.G., Davis, L.R., Willman, J.S., Jones, S.E., Kidd, I.M., Pattison, J.R. & Tyrrell, D.A. (1985) Experimental parvoviral infection in humans. *The Journal of Infectious Diseases*, **152**, 257–265.

Arthur, J.L., Higgins, G.D., Davidson, G.P., Givney, R.C. & Ratcliff, R.M. (2009) A novel bocavirus associated with acute gastroenteritis in Australian children. *PLoS Pathogens*, **5**, e1000391.

Atchison, R.W., Casto, B.C. & Hammon, W.M. (1965) Adenovirus-associated defective virus particles. *Science*, **149**, 754–756.

Baylis, S.A. (2008) Standardization of nucleic acid amplification technique (NAT)-based assays for different genotypes of parvovirus B19: a meeting summary. *Vox Sanguinis*, **94**, 74–80.

Baylis, S.A., Shah, N. & Minor, P.D. (2004) Evaluation of different assays for the detection of parvovirus B19 DNA in human plasma. *Journal of Virological Methods*, **121**, 7–16.

Baylis, S.A., Ma, L., Padley, D.J., Heath, A.B., Yu, M.W.; Collaborative Study Group. (2012) Collaborative study to establish a World Health Organization International genotype panel for parvovirus B19 DNA nucleic acid amplification technology (NAT)-based assays. *Vox Sanguinis*, **102**, 204–211.

Benjamin, L.A., Lewthwaite, P., Vasanthapuram, R., Zhao, G., Sharp, C., Simmonds, P., Wang, D. & Solomon, T. (2011) Human parvovirus 4 as potential cause of encephalitis in children, India. *Emerging Infectious Diseases*, **17**, 1484–1487.

Blumel, J., Eis-Hubinger, A.M., Stuhler, A., Bonsch, C., Gessner, M. & Lower, J. (2005) Characterization of parvovirus B19 genotype 2 in KU812Ep6 cells. *Journal of Virology*, **79**, 14197–14206.

Brown, K.E. (2010) The expanding range of parvoviruses which infect humans. *Reviews in Medical Virology*, **20**, 231–244.

Brown, K.E., Anderson, S.M. & Young, N.S. (1993) Erythrocyte P antigen: cellular receptor for B19 parvovirus. *Science*(New York, N.Y.), **262**, 114–117.

Brown, K.E., Young, N.S., Alving, B.M. & Barbosa, L.H. (2001) Parvovirus B19: implications for transfusion medicine. Summary of a workshop. *Transfusion*, **41**, 130–135.

Burnouf-Radosevich, M., Appourchaux, P., Huart, J.J. & Burnouf, T. (1994) Nanofiltration, a new specific virus elimination method applied to high-purity factor IX and factor XI concentrates. *Vox Sanguinis*, **67**, 132–138.

Candotti, D., Etiz, N., Parsyan, A. & Allain, J.P. (2004) Identification and characterization of persistent human erythrovirus infection in blood donor samples. *Journal of Virology*, **78**, 12169–12178.

Chen, M.Y., Yang, S.J. & Hung, C.C. (2011) Placental transmission of human parvovirus 4 in newborns with hydrops, Taiwan. *Emerging Infectious Diseases*, **17**, 1954–1956.

Chow, B.D., Ou, Z. & Esper, F.P. (2010) Newly recognized bocaviruses (HBoV, HBoV2) in children and adults with gastrointestinal illness in the united states. *Journal of Clinical Virology: The Official Publication of the Pan American Society for Clinical Virology*, **47**, 143–147.

Cossart, Y.E., Field, A.M., Cant, B. & Widdows, D. (1975) Parvovirus-like particles in human sera. *Lancet*, **1**, 72–73.

Csagola, A., Lorincz, M., Cadar, D., Tombacz, K., Biksi, I. & Tuboly, T. (2012) Detection, prevalence and analysis of emerging porcine parvovirus infections. *Archives of Virology*, **157**, 1003–1010.

Dijkman, R., Koekkoek, S.M., Molenkamp, R., Schildgen, O. & van der Hoek, L. (2009) Human bocavirus can be cultured in differentiated human airway epithelial cells. *Journal of Virology*, **83**, 7739–7748.

Don, M., Soderlund-Venermo, M., Valent, F., Lahtinen, A., Hedman, L., Canciani, M., Hedman, K. & Korppi, M. (2010) Serologically verified human bocavirus pneumonia in children. *Pediatric Pulmonology*, **45**, 120–126.

Eis-Hubinger, A.M., Drexler, J.F., Reber, U., Panning, M., Drosten, C., Weber-Schehl, M., Hedges, D., Baylis, S.A. & Roth, W.K. (2010) Absence of detection of novel human parvoviruses in german plasma donations. *Transfusion*, **50**, 266–267.

EMA (2010). Guideline on plasma-derived medicinal products. Document reference: EMA/CHMP/BWP/706271/2010. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2011/07/WC500109627.pdf

Enders, M., Schalasta, G., Baisch, C., Weidner, A., Pukkila, L., Kaikkonen, L., Lankinen, H., Hedman, L., Soderlund-Venermo, M. & Hedman, K. (2006) Human parvovirus B19 infection during pregnancy – value of modern molecular and serological diagnostics. *Journal of Clinical Virology: the Official Publication of the Pan American Society for Clinical Virology*, **35**, 400–406.

Enders, M., Weidner, A., Rosenthal, T., Baisch, C., Hedman, L., Soderlund-Venermo, M. & Hedman, K. (2008) Improved diagnosis of gestational parvovirus B19 infection at the time of nonimmune fetal hydrops. *The Journal of Infectious Diseases*, **197**, 58–62.

European Pharmacopoeia Commission, Council of Europe European Directorate for the Quality of Medicines (2011) Human plasma (pooled and treated for virus inactication), monograph 1646 (01/2011). In: European Pharmacopeia, 7th edn. Council of Europe, Strasbourg.

Freitas, R.B., Melo, F.L., Oliveira, D.S., Romano, C.M., Freitas, M.R., Macedo, O., Linhares, A.C., de A Zanotto, P.M. & Durigon, E.L. (2008) Molecular characterization of human erythrovirus B19 strains obtained from patients with several clinical presentations in the Amazon region of Brazil. *Journal of Clinical Virology: the Official Publication of the Pan American Society for Clinical Virology*, **43**, 60–65.

Frickhofen, N., Abkowitz, J.L., Safford, M., Berry, J.M., Antunez-de-Mayolo, J., Astrow, A., Cohen, R., Halperin, I., King, L. & Mintzer, D. (1990) Persistent B19 parvovirus infection in patients infected with human immunodeficiency virus type 1 (HIV-1): a treatable cause of anemia in AIDS. *Annals of Internal Medicine*, **113**, 926–933.

Fryer, J.F., Kapoor, A., Minor, P.D., Delwart, E. & Baylis, S.A. (2006) Novel parvovirus and related variant in human plasma. *Emerging Infectious Diseases*, **12**, 151–154.

Fryer, J.F., Delwart, E., Bernardin, F., Tuke, P.W., Lukashov, V.V. & Baylis, S.A. (2007a) Analysis of two human parvovirus PARV4 genotypes identified in human plasma for fractionation. *The Journal of General Virology*, **88**, 2162–2167.

Fryer, J.F., Delwart, E., Hecht, F.M., Bernardin, F., Jones, M.S., Shah, N. & Baylis, S.A. (2007b) Frequent detection of the parvoviruses, PARV4 and PARV5, in plasma from blood donors and symptomatic individuals. *Transfusion*, **47**, 1054–1061.

Fryer, J.F., Hubbard, A.R. & Baylis, S.A. (2007c) Human parvovirus PARV4 in clotting factor VIII concentrates. *Vox Sanguinis*, **93**, 341–347.

Grabarczyk, P., Kalinska, A., Kara, M., Wieczorek, R., Ejduk, A., Sulkowska, E., Golebiowska-Staroszczyk, S., Matysiak, M., Baylis, S.A. & Brojer, E. (2011) Identification and characterization of acute infection with parvovirus B19 genotype 2 in immunocompromised patients in Poland. *Journal of Medical Virology*, **83**, 142–149.

Hokynar, K., Soderlund-Venermo, M., Pesonen, M., Ranki, A., Kiviluoto, O., Partio, E.K. & Hedman, K. (2002) A new parvovirus genotype persistent in human skin. *Virology*, **302**, 224–228.

Hokynar, K., Norja, P., Laitinen, H., Palomaki, P., Garbarg-Chenon, A., Ranki, A., Hedman, K. & Soderlund-Venermo, M. (2004) Detection and differentiation of human parvovirus variants by commercial quantitative real-time PCR tests. *Journal of Clinical Microbiology*, **42**, 2013–2019.

Hubschen, J.M., Mihneva, Z., Mentis, A.F., Schneider, F., Aboudy, Y., Grossman, Z., Rudich, H., Kasymbekova, K., Sarv, I., Nedeljkovic, J., Tahita, M.C., Tarnagda, Z., Ouedraogo, J.B., Gerasimova, A.G., Moskaleva, T.N., Tikhonova, N.T., Chitadze, N., Forbi, J.C., Faneye, A.O., Otegbayo, J.A., Charpentier, E. & Muller, C.P. (2009) Phylogenetic analysis of human parvovirus b19 sequences from eleven different countries confirms the predominance of genotype 1 and suggests the spread of genotype 3b. *Journal of Clinical Microbiology*, **47**, 3735–3738.

Jones, M.S., Kapoor, A., Lukashov, V.V., Simmonds, P., Hecht, F. & Delwart, E. (2005) New DNA viruses identified in patients with acute viral infection syndrome. *Journal of Virology*, **79**, 8230–8236.

Kantola, K., Sadeghi, M., Antikainen, J., Kirveskari, J., Delwart, E., Hedman, K. & Soderlund-Venermo, M. (2010) Real-time quantitative PCR detection of four human bocaviruses. *Journal of Clinical Microbiology*, **48**, 4044–4050.

Kantola, K., Hedman, L., Arthur, J., Alibeto, A., Delwart, E., Jartti, T., Ruuskanen, O., Hedman, K. & Söderlund-Venermo, M. (2011) Seroepidemiology of human bocaviruses 1–4. *Journal of Infectious Diseases*, **9**, 1403–1412.

Kapoor, A., Slikas, E., Simmonds, P., Chieochansin, T., Naeem, A., Shaukat, S., Alam, M.M., Sharif, S., Angez, M., Zaidi, S. & Delwart, E. (2009) A newly identified bocavirus species in human stool. *The Journal of Infectious Diseases*, **199**, 196–200.

Kapoor, A., Simmonds, P., Slikas, E., Li, L., Bodhidatta, L., Sethabutr, O., Triki, H., Bahri, O., Oderinde, B.S., Baba, M.M., Bukbuk, D.N., Besser, J., Bartkus, J. & Delwart, E. (2010) Human bocaviruses are highly diverse, dispersed, recombination prone, and prevalent in enteric infections. *The Journal of Infectious Diseases*, **201**, 1633–1643.

Ke, L., He, M., Li, C., Liu, Y., Gao, L., Yao, F., Li, J., Bi, X., Lv, Y., Wang, J., Hirsch, M.L. & Li, W. (2011) The prevalence of human parvovirus B19 DNA and antibodies in blood donors from four Chinese blood centers. *Transfusion*, **51**, 1909–1918.

Keller, L.W., Barbosa, M.L., Melo, F.L., Pereira, L. M., David-Neto, E., Lanhez, L.E. & Durigon, E. L. (2009) Phylogenetic analysis of a near-full-length sequence of an erythrovirus genotype 3 strain isolated in Brazil. *Archives of Virology*, **154**, 1685–1687.

Kishore, J., Srivastava, M. & Choudhary, N. (2010) Standardization of B19 IgG ELISA to study the seroepidemiology of parvovirus B19 in North Indian voluntary blood donors. *Asian Journal of Transfusion Science*, **2**, 86–90.

Kleinman, S.H., Glynn, S.A., Lee, T.H., Tobler, L., Montalvo, L., Todd, D., Kiss, J.E., Shyamala, V. & Busch, M.P.National Heart, Lung, Blood Institute Retrovirus Epidemiology Donor Study (REDS-II) (2007) Prevalence and quantitation of parvovirus B19 DNA levels in blood donors with a sensitive polymerase chain reaction screening assay. *Transfusion*, **10**, 1756–1764.

Koduri, P.R., Kumapley, R., Valladares, J. & Teter, C. (1999) Chronic pure red cell aplasia caused by parvovirus B19 in AIDS: use of intravenous immunoglobulin – a report of eight patients. *American Journal of Hematology*, **61**, 16–20.

Koenigbauer, U.F., Eastlund, T. & Day, J.W. (2000) Clinical illness due to parvovirus B19 infection after infusion of solvent/detergent-treated pooled plasma. *Transfusion*, **40**, 1203–1206.

Koppelman, M.H., Cuypers, H.T., Emrich, T. & Zaaijer, H.L. (2004) Quantitative real-time detection of parvovirus B19 DNA in plasma. *Transfusion*, **44**, 97–103.

Koppelman, M.H., Rood, I.G., Fryer, J.F., Baylis, S. A. & Cuypers, H.T. (2007) Parvovirus B19 genotypes 1 and 2 detection with real-time polymerase chain reaction assays. *Vox Sanguinis*, **93**, 208–215.

Koppelman, M.H., van Swieten, P. & Cuijpers, H. T. (2011) Real-time polymerase chain reaction detection of parvovirus B19 DNA in blood donations using a commercial and an in-house assay. *Transfusion*, **51**, 1346–1354.

Koppelman, M.H., Cuijpers, H.T., Wessberg, S., Valkeajarvi, A., Pichl, L., Schottstedt, V. & Saldanha, J. (2012) Multicenter evaluation of a commercial multiplex polymerase chain reaction test for screening plasma donations for parvovirus B19 DNA and hepatitis A virus RNA. *Transfusion*, **7**, 1498–1508.

Kurtzman, G.J., Cohen, B., Meyers, P., Amunullah, A. & Young, N.S. (1988) Persistent B19 parvovirus infection as a cause of severe chronic anaemia in children with acute lymphocytic leukaemia. *Lancet*, **2**, 1159–1162.

Kurtzman, G., Frickhofen, N., Kimball, J., Jenkins, D.W., Nienhuis, A.W. & Young, N.S. (1989) Pure red-cell aplasia of 10 years' duration due to persistent parvovirus B19 infection and its cure with immunoglobulin therapy. *New England Journal of Medicine*, **321**, 519–523.

Lahtinen, A., Kivela, P., Hedman, L., Kumar, A., Kantele, A., Lappalainen, M., Liitsola, K., Ristola, M., Delwart, E., Sharp, C., Simmonds, P., Soderlund-Venermo, M. & Hedman, K. (2011) Serodiagnosis of primary infections with human parvovirus 4, Finland. *Emerging Infectious Diseases*, **17**, 79–82.

Longhi, E., Bestetti, G., Acquaviva, V., Foschi, A., Piolini, R., Meroni, L., Magni, C., Antinori, S., Parravicini, C. & Corbellino, M.(2007) Human parvovirus 4 in the bone marrow of italian patients with AIDS. *AIDS* (London, England), **21**, 1481–1483.

Lurcharchaiwong, W., Chieochansin, T., Payungporn, S., Theamboonlers, A. & Poovorawan, Y. (2008) Parvovirus 4 (PARV4) in serum of intravenous drug users and blood donors. *Infection*, **36**, 488–491.

Ma, Y.Y., Guo, Y., Zhao, X., Wang, Z., Lv, M.M., Yan, Q.P. & Zhang, J.G. (2012) Human parvovirus PARV4 in plasma pools of Chinese origin. *Vox Sanguinis*, doi: 10.1111/j.1423-0410.2012.01605.x. [Epub ahead of print].

Makris, M., Preston, F.E., Rosendaal, F.R., Underwood, J.C., Rice, K.M. & Triger, D.R. (1996) The natural history of chronic hepatitis C in haemophiliacs. *British Journal of Haematology*, **94**, 746–752.

Makris, M., Calizzani, G., Fischer, K., Gilman, E. A., Hay, C.R.M., Lambert, T., Lassila, R., Ludlam, C.A. & Mannucci, P.M. (2011) EUHASS: The European Haemophilia Safety Surveillance system. *Thrombosis Research*, **127**, 22–25.

Manning, A., Willey, S.J., Bell, J.E. & Simmonds, P. (2007) Comparison of tissue distribution, persistence, and molecular epidemiology of parvovirus B19 and novel human parvoviruses PARV4 and human bocavirus. *The Journal of Infectious Diseases*, **195**, 1345–1352.

Mannucci, P.M. & Tuddenham, E.G. (2001) The hemophilias – from royal genes to gene therapy. *The New England Journal of Medicine*, **344**, 1773–1779.

Meriluoto, M., Hedman, L., Tanner, L., Simell, V., Makinen, M., Simell, S., Mykkanen, J., Korpelainen, J., Ruuskanen, O., Ilonen, J., Knip, M., Simell, O., Hedman, K. & Soderlund-Venermo, M. (2012) Association of human bocavirus 1 infection with respiratory disease in childhood follow-up study, Finland. *Emerging Infectious Diseases*, **18**, 264–271.

Millar, C.M., Connor, N., Dolan, G., Lee, C.A., Makris, M., Wilde, J., Winter, M., Ironside, J.W., Gill, N. & Hill, F.G. (2010) Risk reduction strategies for variant creutzfeldt-jakob disease transmission by UK plasma products and their impact on patients with inherited bleeding disorders. *Haemophilia: The Official Journal of the World Federation of Hemophilia*, **16**, 305–315.

Modrow, S., Wenzel, J.J., Schimanski, S., Schwarzbeck, J., Rothe, U., Oldenburg, J., Jilg, W. & Eis-Hubinger, A.M. (2011) Prevalence of nucleic acid sequences specific for human parvoviruses, hepatitis A and hepatitis E viruses in coagulation factor concentrates. *Vox Sanguinis*, **100**, 351–358.

Mossong, J., Hens, N., Friederichs, V., Davidkin, I., Broman, M., Litwinska, B., Siennicka, J., Trzcinska, A., VAN Damme, P., Beutels, P., Vyse, A., Shkedy, Z., Aerts, M., Massari, M. & Gabutti, G. (2008) Parvovirus B19 infection in five European countries: seroepidemiology, force of infection and maternal risk of infection. *Epidemiology and Infection*, **136**, 1059–1068.

Naides, S.J., Scharosch, L.L., Foto, F. & Howard, E.J. (1990) Rheumatologic manifestations of

human parvovirus B19 infection in adults. initial two-year clinical experience. *Arthiritis and Rheumatism*, **33**, 1297–1309.

Nguyen, Q.T., Sifer, C., Schneider, V., Allaume, X., Servant, A., Bernaudin, F., Auguste, V. & Garbarg-Chenon, A. (1999) Novel human erythrovirus associated with transient aplastic anemia. *Journal of Clinical Microbiology*, **37**, 2483–2487.

Nguyen, Q.T., Wong, S., Heegaard, E.D. & Brown, K.E. (2002) Identification and characterization of a second novel human erythrovirus variant, A6. *Virology*, **301**, 374–380.

Norja, P., Hokynar, K., Aaltonen, L.M., Chen, R., Ranki, A., Partio, E.K., Kiviluoto, O., Davidkin, I., Leivo, T., Eis-Hubinger, A.M., Schneider, B., Fischer, H.P., Tolba, R., Vapalahti, O., Vaheri, A., Soderlund-Venermo, M. & Hedman, K. (2006) Bioportfolio: lifelong persistence of variant and prototypic erythrovirus DNA genomes in human tissue. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 7450–7453.

Norja, P., Hedman, L., Kantola, K., Kemppainen, K., Suvilehto, J., Pitkäranta, A., Aaltonen, L.M., Seppänen, M., Hedman, K. & Söderlund-Venermo, M. (2012) Occurrence of human bocaviruses and parvovirus 4 in solid tissues. *Journal of Medical Virology*, **8**, 1267–1273.

Panning, M., Kobbe, R., Vollbach, S., Drexler, J.F., Adjei, S., Adjei, O., Drosten, C., May, J. & Eis-Hubinger, A.M. (2010) Novel human parvovirus 4 genotype 3 in infants, Ghana. *Emerging Infectious Diseases*, **16**, 1143–1146.

Pattison, J.R., Jones, S.E., Hodgson, J., Davis, L.R., White, J.M., Stroud, C.E. & Murtaza, L. (1981) Parvovirus infections and hypoplastic crisis in sickle-cell anaemia. *Lancet*, **1**, 664–665.

Peden, A., McCardle, L., Head, M.W., Love, S., Ward, H.J., Cousens, S.N., Keeling, D.M., Millar, C.M., Hill, F.G. & Ironside, J.W. (2010) Variant CJD infection in the spleen of a neurologically asymptomatic UK adult patient with haemophilia. *Haemophilia: The Official Journal of the World Federation of Hemophilia*, **16**, 296–304.

Reid, D.M., Reid, T.M., Brown, T., Rennie, J.A. & Eastmond, C.J. (1985) Human parvovirus-associated arthritis: a clinical and laboratory description. *Lancet*, **1**, 422–425.

Richardson, L.C. & Evatt, B.L. (2000) Risk of hepatitis A virus infection in persons with hemophilia receiving plasma-derived products. *Transfusion Medicine Reviews*, **14**, 64–73.

Saal, J.G., Steidle, M., Einsele, H., Muller, C.A., Fritz, P. & Zacher, J. (1992) Persistence of B19 parvovirus in synovial membranes of patients with rheumatoid arthritis. *Rheumatology International*, **12**, 147–151.

Sanabani, S., Neto, W.K., Pereira, J. & Sabino, E.C. (2006) Sequence variability of human erythroviruses present in bone marrow of brazilian patients with various parvovirus B19-related hematological symptoms. *Journal of Clinical Microbiology*, **44**, 604–606.

Schmidt, I., Blumel, J., Seitz, H., Willkommen, H. & Lower, J. (2001) Parvovirus B19 DNA in plasma pools and plasma derivatives. *Vox Sanguinis*, **81**, 228–235.

Schneider, B., Becker, M., Brackmann, H.H. & Eis-Hubinger, A.M. (2004) Contamination of coagulation factor concentrates with human parvovirus B19 genotype 1 and 2. *Thrombosis and Haemostasis*, **92**, 838–845.

Schneider, B., Fryer, J.F., Reber, U., Fischer, H.P., Tolba, R.H., Baylis, S.A. & Eis-Hubinger, A.M. (2008a) Persistence of novel human parvovirus PARV4 in liver tissue of adults. *Journal of Medical Virology*, **80**, 345–351.

Schneider, B., Fryer, J.F., Oldenburg, J., Brackmann, H.H., Baylis, S.A. & Eis-Hubinger, A.M. (2008b) Frequency of contamination of coagulation factor concentrates with novel human parvovirus PARV4. *Haemophilia: The Official Journal of the World Federation of Hemophilia*, **14**, 978–986.

Servant-Delmas, A., Laperche, S., Mercier, M., Elghouzzi, M.H., Lionnet, F., Stankovic, K., Girot, R. & Lefrere, J.J. (2009) Human parvovirus 4 in recipients of cellular products and in blood donors: epidemiologic similarity with B19 parvovirus. *Transfusion*, **49**, 1771–1773.

Sharp, C.P., Lail, A., Donfield, S., Simmons, R., Leen, C., Klenerman, P., Delwart, E., Gomperts, E.D. & Simmonds, P. (2009) High frequencies of exposure to the novel human parvovirus PARV4 in hemophiliacs and injection drug users, as detected by a serological assay for PARV4 antibodies. *The Journal of Infectious Diseases*, **200**, 1119–1125.

Sharp, C.P., Lail, A., Donfield, S., Gomperts, E.D. & Simmonds, P. (2012) Virologic and clinical features of primary infection with human parvovirus 4 in subjects with hemophilia: frequent transmission by virally inactivated clotting factor concentrates. *Transfusion*, **52**, 1482–1489.

Siegl, G. & Cassinotti, P. (1998) Presence and significance of parvovirus B19 in blood and blood products. *Biologicals: Journal of the International Association of Biological Standardization*, **26**, 89–94.

Simmonds, P., Manning, A., Kenneil, R., Carnie, F.W. & Bell, J.E. (2007) Parenteral transmission of the novel human parvovirus PARV4. *Emerging Infectious Diseases*, **13**, 1386–1388.

Simmonds, P., Douglas, J., Bestetti, G., Longhi, E., Antinori, S., Parravicini, C. & Corbellino, M. (2008) A third genotype of the human parvovirus PARV4 in sub-saharan africa. *The Journal of General Virology*, **89**, 2299–2302.

Soderlund, M., von Essen, R., Haapasaari, J., Kiistala, U., Kiviluoto, O. & Hedman, K. (1997) Persistence of parvovirus B19 DNA in synovial membranes of young patients with and without chronic arthropathy. *Lancet*, **349**, 1063–1065.

Soderlund-Venermo, M., Hokynar, K., Nieminen, J., Rautakorpi, H. & Hedman, K. (2002) Persistence of human parvovirus B19 in human tissues. *Pathologie-Biologie*, **50**, 307–316.

Soderlund-Venermo, M., Lahtinen, A., Jartti, T., Hedman, L., Kemppainen, K., Lehtinen, P., Allander, T., Ruuskanen, O. & Hedman, K. (2009) Clinical assessment and improved diagnosis of bocavirus-induced wheezing in children, Finland. *Emerging Infectious Diseases*, **15**, 1423–1430.

Soucie, J.M., Erdman, D.D., Evatt, B.L., Anderson, L.J., Torok, T.J., El-Jamil, M., Barnhart, E., Tepper, M., Burrill, H.N., Pickett, A.M. & Mengeling, W.L. (2000) Investigation of porcine parvovirus among persons with hemophilia receiving hyate: C porcine factor VIII concentrate. *Transfusion*, **40**, 708–711.

Soucie, J.M., De Staercke, C., Chitlu, R.M.B., Gruppo, R.A., Hooper, W.C., Kessler, C.M., Roshni Kulkarni, R., Manco-Johnson, M.J., Monahan, P.E., Oakley, Meredith., Powell, J., Pyle, M., Recht, M., Riske, B., Sabio, H. & Trimble, S. (2011) Continued Transmission of Parvovirus B19 in Plasma-Derived Factor Concentrates After the Implementation of B19 Nucleic Acid Plasma Minipool Screening. *Blood (ASH Annual Meeting Abstracts)*, **118**, 3378.

Szelei, J., Liu, K., Li, Y., Fernandes, S. & Tijssen, P. (2010) Parvovirus 4-like virus in blood products. *Emerging Infectious Diseases*, **16**, 561–564.

Szomor, K.N., Kapusinszky, B., Rigo, Z., Kis, Z., Rozsa, M., Farkas, A., Szilagyi, A., Berencsi, G. & Takacs, M. (2009) Detection of human bocavirus from fecal samples of Hungarian children with acute gastroenteritis. *Intervirology*, **52**, 17–21.

Touinssi, M., Brisbarre, N., Picard, C., Frassati, C., Dussol, B., Uch, R., Gallian, P., Cantaloube, J.F., Micco, P. & Biagini, P. (2010) Parvovirus 4 in blood donors, France. *Emerging Infectious Diseases*, **16**, 165–166.

Vallerini, D., Barozzi, P., Quadrelli, C., Bosco, R., Potenza, L., Riva, G., Gregorini, G., Sandrini, S., Tironi, A., Montagnani, G., De Palma, M., Torelli, G., Delwart, E. & Luppi, M. (2008) Parvoviruses in blood donors and transplant patients, Italy. *Emerging Infectious Diseases*, **14**, 185–186.

White, D.G., Woolf, A.D., Mortimer, P.P., Cohen, B.J., Blake, D.R. & Bacon, P.A. (1985) Human parvovirus arthropathy. *Lancet*, **1**, 419–421.

Willkommen, H., Schmidt, I. & Lower, J. (1999) Safety issues for plasma derivatives and benefit from NAT testing. *Biologicals: Journal of the International Association of Biological Standardization*, **27**, 325–331.

Yu, J.M., Li, D.D., Xu, Z.Q., Cheng, W.X., Zhang, Q., Li, H.Y., Cui, S.X., Miao-Jin, ??., Yang, S.H., Fang, Z.Y. & Duan, Z.J. (2008) Human bocavirus infection in children hospitalized with acute gastroenteritis in China. *Journal of Clinical Virology: The Official Publication of the Pan American Society for Clinical Virology*, **42**, 280–285.

Yu, X., Zhang, J., Hong, L., Wang, J., Yuan, Z., Zhang, X. & Ghildyal, R. (2012) High prevalence of human parvovirus 4 infection in HBV and HCV infected individuals in shanghai. *PLoS ONE*, **7**, e29474.